

09/937,187

## WEST Search History

DATE: Thursday, June 19, 2003

Set Name Query  
side by sideHit Count Set Name  
result set

DB=USPT,DWPI; PLUR=YES; OP=OR

L24	(phage\$)near20(selenium)	0	L24
L23	L22 and (phage\$ or bacteriophage\$)	1	L23
L22	L19 near30 (phage or viral or coat\$ or fusion\$)	3	L22
L21	L20 and (amplif\$ or librar\$)	55	L21
L20	L19 and (phage or viral or coat\$)	97	L20
L19	l15 not L18	199	L19
L18	secy	110	L18
L17	L15 near30 coat\$	1	L17
L16	L15 and coat\$	117	L16
L15	SeCys or selenocys\$	309	L15
L14	L12 and selenocys\$	0	L14
L13	L12 and SeCys	0	L13
L12	L11 and (fusion or fused)near30(coat\$)	56	L12
L11	(seleno or Se)near2(cys or cystein\$)	1230	L11
L10	fusion near10 l2	3	L10
L9	L2 near10000 phage\$	0	L9
L8	L2 near1000 phage\$	0	L8
L7	L2 near100 phage\$	0	L7
L6	L2 near50 phage\$	0	L6
L5	L2 near30 phage\$	0	L5
L4	L2 near20 phage\$	0	L4
L3	L2 and phage\$	59	L3
L2	L1 or (seleno)near2(cys or cystein\$)	132	L2
L1	SeCys	110	L1

END OF SEARCH HISTORY

See 5,187,078

**WEST**

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L28: Entry 4 of 5

File: USPT

Feb 16, 1993

DOCUMENT-IDENTIFIER: US 5187078 A

TITLE: Plasma-type glutathione peroxidase gene and application of the same

Brief Summary Text (7):

Glutathione peroxidase is a protein containing selenium, which exists in the active site in the form of selenocystein (Sec). The opal codon TGA, which normally is a termination codon in a DNA sequence, codes for Sec in a cloned glutathione peroxidase gene derived from mouse [EMBO Journal, vol 5, No. 6, 1221-1227 (1986)].

Brief Summary Text (16):

Triplet codons for a 13 amino acid sequence which is a portion of the polypeptide amino acid sequences thus determined were estimated, from which a probe mixture of 39 anti-codon bases was prepared. This DNA probe was used to screen a cDNA library of .lambda.-gt11 phage vector which was prepared from human placenta mRNA, thus identifying a gene consisting of 1,603 bases and encoding 225 amino acid residues. Analysis of the DNA sequence and the amino acid sequence proved that the selenocystein which is a site characteristic to glutathione peroxidase was encoded by an opal codon TGA, and the amino acid sequence in the neighborhood of the active center was -Ala-Ser-Tyr-\*\*\*-Gly-Leu-Thr-, wherein \*\*\* denotes a selenocystein residue. The amino acid sequence had a homology of only about 30% with that of h-e.GSHPx, and the homology of the base sequence with that of h-e.GSHPx was only about 25%. Furthermore, the h-p.GSHPx was found to have in its N-terminal side, an amino acid sequence of the formula -Ile-Ser-Gly-Thr-Ile-, and in its C-terminal side, an amino acid sequence of the formula -Leu-Gly-Thr-Ser-Asp-. This gene DNA was recombined into an expression vector and transformed, for example, in monkey kidney cells (COS) to express a homotetramer which comprises a sub-unit having a molecular weight of 23,000.+-.2,000 and which could be discharged outside the cells by the action of the signal peptide which is characteristic to the amino acid sequence on the N-terminal side. The present inventors have thus prepared the h-p.GSHPx as well as its gene DNA, and have established a process for its preparation.

Brief Summary Text (18):

Accordingly, an object of this invention is to provide a DNA fragment comprising a base sequence coding for a polypeptide which is h-p.GSHPx, which polypeptide comprises, in a neighborhood of the polypeptide active center, amino acid sequence (I) of the formula -Ala-Ser-Tyr-\*\*\*-Gly-Leu-Thr-, wherein \*\*\* represents a selenocystein residue, in the N-terminal side of amino acid sequence (I), an amino acid sequence of the formula -Ile-Ser-Gly-Thr-Ile-, and in the C-terminal side of amino acid sequence (I), an amino acid sequence of the formula -Leu-Gly-Thr-Ser-Asp-; said h-p.GSHPx comprising a sub-unit having a molecular weight of 23,000.+-.2,000, having glutathione as a substrate, and exhibiting glutathione peroxidase activity which catalyses a reaction converting two moles of glutathione and one mole of hydrogen peroxide into two moles of glutathione oxide and two moles of water.

Detailed Description Text (3):

The h-p.GSHPx gene of the present invention may be prepared using a commercially available human placenta cDNA library or a library obtained by incorporating mRNA prepared from human placenta tissue into a vector. For example, a powdered human placenta tissue is homogenized with a guanidium solution to produce a suspension. The suspension is passed several times through a 18.5 gauge injection needle to fragment the high molecular DNA, multi-layered onto a 5.7 M cesium chloride solution, and centrifuged overnight at 25.degree. C. and at 35,000 rpm. The precipitate is dissolved

into water, treated with an equivalent amount of phenol-chloroform, followed by an addition of sodium acetate in an amount to make its concentration 0.3 M and by a further addition of 2.5-volume ethanol, thus effecting re-precipitation. The total RNA is then collected by centrifugation. The RNA is dissolved into water, heated for 5 minutes at 65.degree. C. and quenched, following which a buffer containing SDS, EDTA, and NaCl is added. The mixture is charged into an oligo(dT)-cellulose column to elute only RNA possessing poly-A-tail. A first chain is synthesized from this poly(A).sup.+ RNA using dNTPs (a mixture of equivalent amounts of dATP, dGTP, dCTP, and dTTP), oligo (dT), and a reverse transcriptase, then a second chain is prepared using RNaseH and DNA polymerase I. The Eco RI site existing within the gene is methylated with Eco RI methylase, the blunt ends are produced using T.sub.4 DNA polymerase, and Eco RI linker is attached to the both ends, followed by the digestion with restriction endonuclease Eco RI and the size fractionation by electrophoresis to remove the surplus linker. The product is incorporated into phage vector .lambda. -gt11, thus producing a cDNA library in which mRNA prepared from placenta tissue is incorporated into the .lambda.gtl1 vector. This cDNA library can be used for preparing a library in which mRNA prepared from human placenta tissue is incorporated into the .lambda.gtl1 vector. A probe consisting of several tens of bases may then be synthesized with reference to a known glutathione peroxidase. For simplicity, screening can be carried out using a synthesized oligonucleotide of a 39-base anti-codon corresponding to a peptide containing TGA coding for selenocystein (Sec) or its downstream peptide, e.g. -Gly-Leu-Thr-Gly-Gln-Tyr-Ile-Glu-Leu-Asn-Ala-Leu-Gln-. For the screening, a host microorganism, e.g. Escherichia coli. LE392, is infected with a library prepared from .lambda.gtl1 vector and lyzed by the plaque hybridization method on an agar medium, and the phage is absorbed in a nylon membrane put onto the lyzed medium surface. The membrane is treated with alkali to denature the DNA. After neutralization, DNA is fixed by drying at 80.degree. C. for 2 hours, the membrane is incubated at 37.degree. C. for 4 hours in a pre-hybridization solution, e.g. a solution containing 5.times.SCC (1-fold; 150 mM NaCl, 15 mM sodium citrate), 5.times.Denhart solution, 50 mM sodium phosphate (pH 6.5), 0.1% SDS (sodium lauryl sulfate), 250 .mu.g/ml non-homological DNA, e.g. salmon sperm DNA, and 20% formamide. To this solution the DNA probe of which the 5'-end of the DNA is labeled with .sup.32 P as described above is added, followed by hybridization at 37.degree. C. overnight. After this, the membrane is washed three times with 2.times.SSC, 0.1% SDS at room temperature, again washed with the same solution at 37.degree. C. for 10 minutes, dried in the air, and subjected to autoradiograph to recover plaques existing at the place where signals appear. The plaque is again charged into the plate to purify it and to screen phage clones comprising the target h-p.GSHPx gene. The purified phage thus screened is then used for the infection of the host organism, and the latter is cultured in a medium overnight. The culture broth is centrifuged to collect the supernatant. To the supernatant are added DNaseI and RNaseA, then the equivalent amount of 20% polyethylene glycol and 2.5 M NaCl, and the mixture is cooled, and centrifuged. The precipitate is suspended in SM (0.1 M NaCl/8 mM MgSO.sub.4 /50mM Tris pH 7.5/0.01% gelatin solution). An equivalent amount of chloroform is added to the suspension, followed by centrifugation. Onto the mixture a water layer of which the density is adjusted to 1.6-1.4 by an addition of cesium chloride is layered and centrifuged. A band containing a phage having a density of 1.6-1.4 is collected, treated with protease, and the DNA is extracted with phenol. The extracted phage DNA is dissolved into a solution containing RNaseA and digested with restriction endonuclease Eco RI to obtain a fragment containing h-p.GSHPx gene of about 1.6 Kbp, which is then collected by gel electrophoresis. FIG. 1 shows a map showing the restriction endonuclease sites of this fragment containing the h-p.GSHPx gene of about 1.6 Kbp.

#### Detailed Description Text (6):

The DNA of h-p.GSHPx which is the target product of the present invention has, for example, a base sequence represented by SEQ ID No. 1. shown later in this specification. This novel DNA has an opal codon TGA coding for selenocystein (Sec) and encodes a polypeptide consisting of 225 amino acid residues, and the amino acid sequence has in the neighborhood of its active center, amino acid sequence (I) of the formula -Ala-Ser-Tyr-\*\*\*-Gly-Leu-Thr-, wherein \*\*\* represents a selenocystein residue, in the N-terminal side of amino acid sequence (I), an amino acid sequence of the formula -Ile-Ser-Gly-Thr-Ile-, and in the C-terminal side of amino acid sequence (I), an amino acid sequence of the formula -Leu-Gly-Thr-Ser-Asp-. In this gene DNA, the 5'-end which is in the upstream of GCC (Ala) may have any codon so long as the same codes for an amino acid. In addition, the 5'-end side may have one or more codons

encoding an amino acid, preferably ATG. It may further be recombined with a polydeoxyribonucleic acid corresponding to a suitable signal peptide. A codon in the 3'-end which is the downstream of AAG (Lys) may be a translational termination codon or any codon encoding an amino acid or a peptide. In addition, there can be one or more codons encoding an amino acid or a peptide at the 3'-end side, provided that in this instance it is desirable that a translational termination codon, e.g. TAA, be present at the 3'-end of these codons.

Detailed Description Text (42):

The amino acid sequence, -Ala-Ser-Tyr-\*\*\*-Gly-Leu-Thr-, wherein \*\*\* denotes a selenocystein residue, existing in the neighborhood of the active center of h-p.GSHPx gene encoded by the base sequence 5'-GCCAGCTACTGAGGCCTGACG-3' (bases 208-228 when the base A of the initiating codon ATG is taken as base 1) was confirmed by the digestion with restriction endonucleases Hinc II and Eco RI of said DNA gene, which produced a DNA fragment of 202-228 bases of the sequence 5'-AACGTGGCCAGCTACTGAGGCCTGACG-3', in which the codon frame starts from the first base A, which correspond to the amino acid sequence, -Asn-Val-Ala-Ser-Tyr-Sec-Gly-Leu-Thr-. In the same way, the amino acid sequence Ile-Ser-Gly-Thr-Ile, which is in the N-terminal side of said amino acid sequence in the neighborhood of the active center of h-p.GSHPx gene encoded by the base sequence 5'-ATAAGTGGCACCATT-3' (bases 106-120 when the base A of the initiating codon ATG is taken as base 1) was confirmed by the digestion with restriction endonucleases Bgl I and Ban II of said DNA gene, which produced a DNA fragment of 102-131 bases of the sequence 5'-TGGCATAAGTGGCACCATTACGAGTACGG-3', in which the codon frame starts with second base G, which corresponds to an amino acid sequence of -Gly-Ile-Ser-Gly-Thr-Ile-Tyr-Glu-Tyr-Gly-. Furthermore, the amino acid sequence -Leu-Gly-Thr-Ser-Asp-, which is in the C-terminal side of said amino acid sequence in the neighborhood of the active center of h-p.GSHPx gene encoded by the base sequence 5'-CTGGGTACATCTGAC-3' (bases 487-501 when the base A of the initiating codon ATG is taken as base 1) was confirmed by the digestion with restriction endonucleases Sac I and Mnl I of said DNA gene, which produced a DNA fragment of 485-514 bases of the sequence 5'-TCCTGGGTACATCTGACCGCCTCTTCTGGG-3', in which the codon frame starts with the third base C, which corresponds to an amino acid sequence of -Leu-Gly-Thr-Ser-Asp-Arg-Leu-Phe-Trp-.

**WEST**[Generate Collection](#)[Print](#)**Search Results - Record(s) 1 through 3 of 3 returned.**☐ 1. Document ID: US 6303295 B1

L22: Entry 1 of 3

File: USPT

Oct 16, 2001

US-PAT-NO: 6303295

DOCUMENT-IDENTIFIER: US 6303295 B1

TITLE: Selenoproteins, coding sequences and methods

DATE-ISSUED: October 16, 2001

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Taylor; Ethan Will	Athens	GA		
Nadimpalli; Ram Gopal	Athens	GA		
Ramanathan; Chandra Sekar	Athens	GA		

US-CL-CURRENT: 435/6; 530/350, 530/400, 536/23.1, 536/23.74

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Claims	KIMC	Draw Desc
Image												

☐ 2. Document ID: US 5830673 A

L22: Entry 2 of 3

File: USPT

Nov 3, 1998

US-PAT-NO: 5830673

DOCUMENT-IDENTIFIER: US 5830673 A

TITLE: Bioassay of selenium

DATE-ISSUED: November 3, 1998

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Engelberg-Kulka; Hanna	Jerusalem			IL
Rechtes; Myriam	Mevasseret Zion			IL

US-CL-CURRENT: 435/7.9; 435/190, 435/207, 435/252.3, 435/252.33, 435/26, 435/320.1, 536/23.2, 536/23.4

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Claims	KIMC	Draw Desc
Image												

☐ 3. Document ID: US 6303295 B1

L22: Entry 3 of 3

File: DWPI

Oct 16, 2001

DERWENT-ACC-NO: 2002-024734

DERWENT-WEEK: 200203

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TITLE: New selenoprotein for use in detecting certain viruses, e.g. human immunodeficiency virus (HIV) or Ebola, cancer and immune system disorders

INVENTOR: NADIMPALLI, R G; RAMANATHAN, C S ; TAYLOR, E W

PRIORITY-DATA: 1996US-0679493 (July 12, 1996), 1995US-001203P (July 14, 1995), 1995US-003112P (September 1, 1995)

## PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
US 6303295 B1	October 16, 2001		202	C12Q001/68

INT-CL (IPC): C12 Q 1/68

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	KIMC	Draw Desc
Image											

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Terms	Documents
L19 near30 (phage or viral or coat\$ or fusion\$)	3

**Display Format:**

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04/937, 187

## WEST Search History

DATE: Thursday, June 19, 2003

<u>Set Name</u>	<u>Query</u>	<u>Hit Count</u>	<u>Set Name</u>
side by side			result set
<i>DB=USPT,DWPI; PLUR=YES; OP=OR</i>			
L28	L27 and l19	5	L28
L27	(librar\$)near20(bacteriophag\$ or phage\$)	7713	L27
L26	L25 and (bacteriophag\$ or phage\$)	34	L26
L25	L19 and librar\$	42	L25
L24	(phage\$)near20(selenium)	0	L24
L23	L22 and (phage\$ or bacteriophage\$)	1	L23
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L16	L15 and coat\$	117	L16
L15	SeCys or selenocys\$	309	L15
L14	L12 and selenocys\$	0	L14
L13	L12 and SeCys	0	L13
L12	L11 and (fusion or fused)near30(coat\$)	56	L12
L11	(seleno or Se)near2(cys or cystein\$)	1230	L11
L10	fusion near10 l2	3	L10
L9	L2 near10000 phage\$	0	L9
L8	L2 near1000 phage\$	0	L8
L7	L2 near100 phage\$	0	L7
L6	L2 near50 phage\$	0	L6
L5	L2 near30 phage\$	0	L5
L4	L2 near20 phage\$	0	L4
L3	L2 and phage\$	59	L3
L2	L1 or (seleno)near2(cys or cystein\$)	132	L2
L1	SeCys	110	L1

END OF SEARCH HISTORY